Sea Monkey Business: What Species Of Artemia Are You Hatching?

Nikki Curreri, [1]Angelina Dilbaryan, [1]Brandon Thomas and [1]Katalin Torok
Mentor: [1]Virginia Sullivan Rachel Carson High School for Coastal Studies

Abstract

There are many distinct species of Artemia. In this Urban Barcoding Project, we seek to investigate if all brine shrimp eggs (cysts) sold in local pet shops and aquarium supply stores are the same species of Artemia or do they vary. Since our school depends on raising Artemia for the success of our horseshoe crab program, we hope to find out this genetic information so that we can later investigate if there are any correlations between hatch rate and species. To conduct this experiment, we hatched Artemia cysts and samples of the nauplii were extracted for DNA collection. The extractions and amplifications were performed during Saturday school at Rachel Carson High School using the footlocker kits borrowed from the Harlem DNA Lab. According to our DNA Subway results, we hatched *Artemia franciscana*. Our data also shows that possibly a combination of *Artemia parthenogenetica* and *Artemia urmiana* are used for instant brine shrimp.

Introduction

In general, most people buying Artemia are not focused on keeping them as pets, but instead are raising them as a form of nutrition for their marine aquariums. In Rachel Carson High School, the ninth grade science research students raise Artemia every week to feed to our horseshoe crabs. As a conservation effort, students in our school raise horseshoe crabs beginning in the fall semester which are then released in the spring as part of a raise and re-nest program to help horseshoe crab population counts. The horseshoe crabs are primarily fed brine shrimp, officially known as Artemia. Throughout last year, the ninth grade students hatched various brands of brine shrimp eggs and found that the hatch rates between brands varied. Based on this observation, we propose to use DNA barcoding to identify which species of Artemia are being sold by different manufacturers.

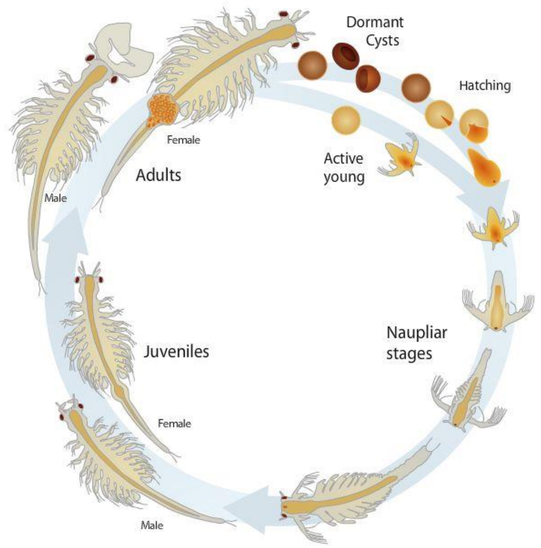
In this Urban Barcoding Project, we seek to investigate if all brine shrimp eggs (cysts) sold in local pet shops and aquarium supply stores are the same species of Artemia or do they vary. Since our school depends on raising Artemia for the success of our horseshoe crab program, we hope to find out this genetic information so that we can later investigate if there are any correlations between hatch rate and species. This year our school expanded the horseshoe crab program so now three high schools in Brooklyn are involved in this conservation effort and the results of this barcoding project could have an impact on all three schools. Furthermore, we would like to barcode one package of Sea Monkeys to determine if they are similar or different from the brine shrimp that we are currently hatching.

Materials & Methods

We ordered our Artemia samples from Amazon.com based on customer reviews. Each sample was photographed an uploaded to the UBP website. The cysts were hatched according to the package instructions. Once hatched, the hatched naupplii were transferred to centrifuge tubes in order to extract the DNA. To do this we added 300µl lysis solution and using a sterile pestle; the samples were mixed and or crushed. The samples were incubated at sixty five degrees Celsius for ten minutes and then the tubes were centrifuged for one minute, allowing the components to separate and the DNA was located in the supernatant. Next, 150µl supernatant was transferred to a new micro centrifuge tube and 3µl silica resin was mixed with the supernatant, allowing the DNA to bind to the silica resin. The tubes were then heated to fifty seven degrees Celsius for five minutes, and then centrifuged for 30 seconds. The supernatant was removed and the silica resin was washed twice with 500µl of cold wash buffer. After the second washing, the tubes were centrifuged and supernatant was removed. 100µl of distilled water was added to the silica in each tube, allowing the nucleic acids to elute from the silica resin. The distilled water, now containing the DNA was incubated for five minutes at fifty seven degrees Celsius. The tubes were centrifuged for 30 seconds and then 90 microliters of the supernatant, containing the DNA was removed and placed in fresh 1.5ml tubes. The tubes were stored on ice until the amplification step. To amplify the samples we mixed 10.5µl of invertebrate primer and 12.5µl of Taq 2x Master mix in a new PCR tube. We then pipetted 2µl of DNA into the appropriate labeled tubes and began thermal cycling using the Eppendorf Thermo cycler and the appropriate barcode program. After thermal cycling, the PCR products were analyzed by Gel electrophoresis and the bands were observed using a UV transilluminator. The samples with observable bands were sent for sequencing. The sequenced samples were analyzed using DNA subway and a BLAST search.

Results

Brine Shrimp Eggs/cysts Brand	Organismal DNA Identified
Ocean Nutrition Instant Brine Shrimp Eggs	*Artemia parthenogenetica* *Artemia urmiana*
Ocean Star International Brine Shrimp Eggs	No DNA extracted
Your Fish Stuff Brine Shrimp Eggs	*Artemia franciscana*
San Francisco Bay Brine Shrimp Eggs	No DNA Extracted
Dr. G's Artemia Kit	*Artemia Franciscana*
Sea Monkeys	No DNA extracted



Discussion

We set out to find what species of Artemia were being sold as brine shrimp eggs. According to DNA subway, two of the brands of brine shrimp eggs were identified as Artemia *franciscana*. One of the brands which is a type of instant brine shrimp was identified with zero mismatches as *Artemia parthenogenetica* and as *Artemia urmiana* but with 18 mismatches. When researching these species of brine shrimp, we discovered that there are currently only two places in Portugal where native *Artemia parthenogenetica* can be found because *Artemia franciscana*, an invasive species, eradicated all other populations resulting in loss of Artemia biodiversity in the Mediterranean region (Pinto, 2013). With our results and this new knowledge, it makes sense that the majority of brine shrimp eggs sold are *Artemia franciscana*. Unfortunately, we were not able to extract DNA samples from three of the brands tested. Although the Sea Monkeys hatched, only two naupliis were visible and therefore the sample may have been too small, and therefore our extraction technique didn't work. The other two brands of brine shrimp eggs simply did not hatch. This may have been due to multiple factors including age of cysts, salinity of water or aeration. Artemia cysts are very durable and can protect the unhatched embryo for up to twenty five years. We tried to extract DNA from the unhatched cysts, but found that the combination of lysis solution and grinding with the pestle, did not break open the tough outer coating, preventing the DNA from being extracted. This information is useful because now that we know that we have access to different species of Artemia. By knowing this information, we can do future studies comparing our horseshoe crab growth if the horseshoe crabs are fed diets of *Artemia franciscana* vs. *Artemia parthenogenetica/Artemia urmiana*. Since we are trying to raise horseshoe crabs for re-nesting purposes, feeding them an optimal diet would help with our conservation efforts.

References

All About Sea Monkeys. Retrieved from https://www.filtersfast.com/articles/All-About-Sea-Monkeys.php

Croghan, P.C. (1958). The Survival of *Artemia Salina* (L.) in Various Media Journal of Experimental Biology 1958 35: 213-218. Retrieved from http://jeb.biologists.org/content/35/1/213

Pinto, P.M., Bio, A., Hontoria, F., Almeida, V., Vieira, N. (2013) Portuguese native *Artemia parthenogenetica* and *Artemia franciscana* survival under different abiotic conditions Journal of Experimental Marine Biology and Ecology vol 440, p. 81-89

Van Stappen, G. (1996) Manual on the Production and Use of Live Food for Aquaculture. Laboratory of Aquaculture & Artemia Reference Center University of Belgium. Retrieved from http://www.fao.org/docrep/003/w3732e/w3732e0m.htm

Acknowledgements

Thank you to Melissa Lee and Christine Marizzi at the Harlem DNA Lab for loaning us the footlocker kit and for helping us with preparation for sequencing. We would also like to thank Dr. Tanacredi, Sixto Portilla and CERCOM for inspiring this project, through our horseshoe crab conservation work.